The Veliger 29(1):48-52 (July 1, 1986)

THE VELIGER

Preservation Artifacts and Their Effects on the Study of Euthecosomatous Pteropod Mollusks

by

RONALD W. GILMER

Department of Biology, Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543, U.S.A.

Abstract. Inaccurate anatomical observations based on preserved specimens of euthecosomatous pteropod mollusks are shown to result from artifacts of preservation. All "aberrant" forms previously described in the literature (including the "minute" and "skinny" stages) can be induced in the laboratory by the addition of preservatives to normal living animals. That these aberrant forms have never been observed in nature further supports the contention that they are preservation artifacts.

INTRODUCTION

PROPER PRESERVATION of delicate zooplanktonic organisms is a troublesome and sophisticated art. The effects of preservation on anatomical structures are often hard to interpret, particularly if living specimens have not been studied, and while one preservation method may work well for a particular species, it can have quite different effects on closely related species (GOHAR, 1937; RUNHAM *et al.,* 1965; UNESCO, 1976). Through necessity, oceanic zooplankton samples are often collected and preserved months or years before they are analyzed and, therefore, knowledge of preservation artifacts is particularly important. The routine use of formaldehyde, added without prior relaxation of specimens, is not satisfactory for preserving many of the more delicate zooplanktonic species (UNESCO, 1976).

A number of disproportionately small "minute" and "aberrant" individuals have been reported in adult-sized shells of preserved members of the family Cavoliniidae (BONNEVIE, 1913; TESCH, 1946; SPOEL, 1962, 1967, 1979, and references cited therein; PAFORT-VAN IERSEL, 1982; PAFORT-VAN IERSEL & SPOEL, 1979; LEYEN & SPOEL, 1982). Rather than regarding these so-called minute forms as fixation artifacts, the works cited above have regarded them as natural stages in the life cycles of cavoliniids. In attempting to explain how these very small individuals could secrete such large shells, SPOEL (1967) suggested that they whirl around inside their disproportionately large shell, adding new shell layers until the fully formed adult shell is completed. SPOEL (1967) further discounts the possibility that the mantle accounts for shell secretion (WILBUR & SALEUDDIN, 1983) by pointing out that the mantle cannot possibly reach to the upper margins of the shell. These assertions are contrary to the results of other workers who have examined shell development in thecosomes (BÉ *et al.,* 1972). More recently, SPOEL (1973, 1979) and PAFORT-VAN IERSEL (1982), in an attempt to explain the "aberrant" morphological forms found in preserved specimens of *Clio pyramidata* Linnaeus (Figure 1b), have suggested that some pteropods reproduce asexually by a scyphozoan-like strobilization process.

There are no reported observations of living aberrant forms and I know of no studies that have seriously examined whether any of these "aberrations" in morphology are based on preservation artifacts. The data presented here give evidence that the proposed "minute" and "aberrant" developmental stages and the process of "asexual strobilization" in cavoliniid pteropods are based entirely on preservation artifacts.

MATERIALS AND METHODS

Various relaxants and fixatives were used in the preservation of live euthecosome pteropods. Animals were either collected by hand using glass jars while SCUBA diving or taken alive from net samples. How formaldehyde affects the contraction of live animals was observed using specimens of *Clio pyramidata* (Figures 1a, b, 2). Animals were preserved directly in 5% formaldehyde without prior relaxing and then examined. They were then dried to constant weight at 80°C for 48 h and used for dry weight and dimensional comparisons. The entire animal and shell were cooled and stored in a desiccator over silica gel and then weighed on a Mettler micro-gram A balance. The soft parts were then removed by crushing the shell and

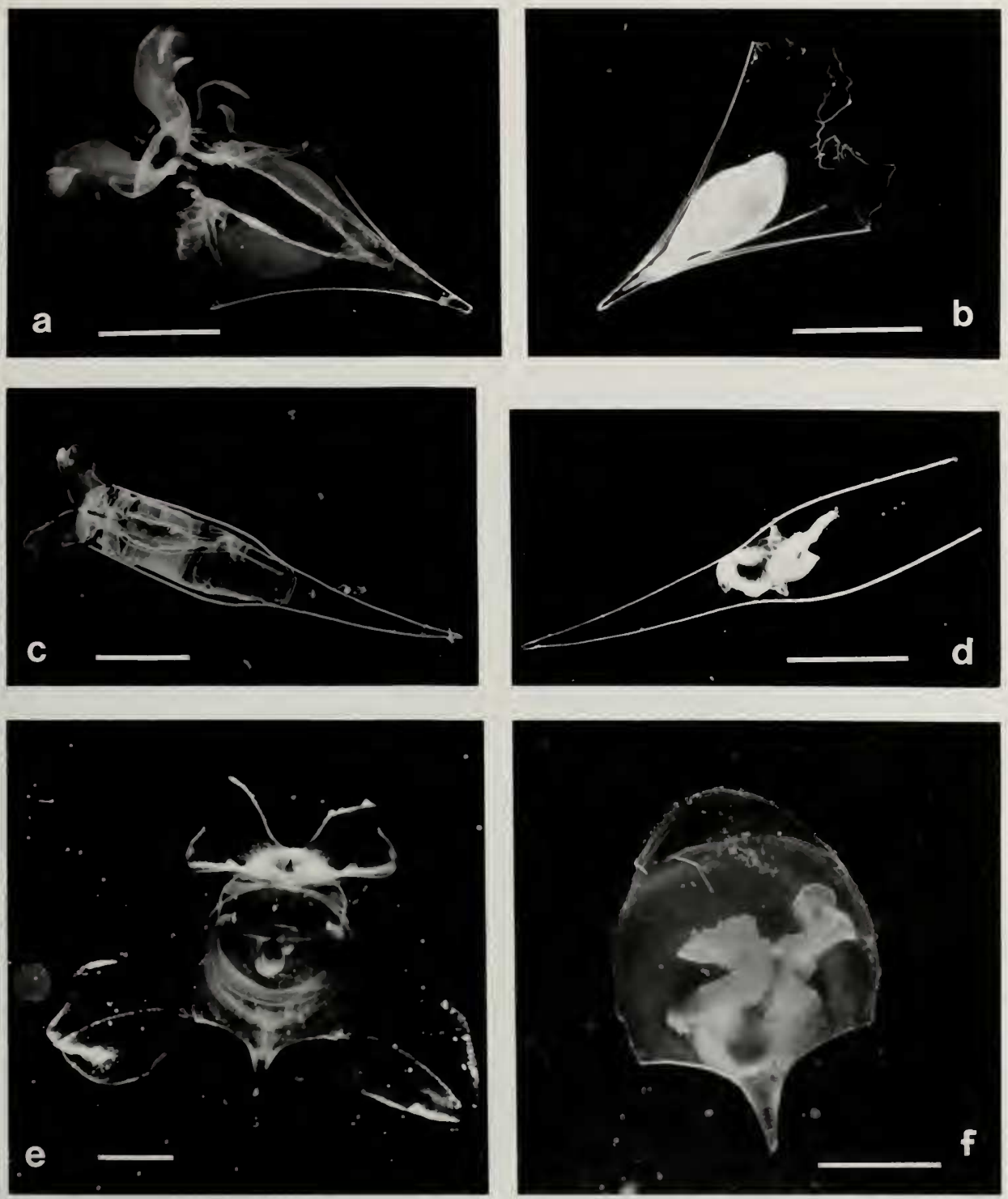

Figure 1

Effects of adding 5% buffered formaldehyde to living adult specimens of thecosome pteropods. a, live *Clio pyramidata*. b, the same animal as "a" after preservation; compare with PAFORT-VAN IERSEL (1982:pl. I). c, live *Cuvierina columnella*. d, the same animal as "c" after preservation. e, live *Cavolinia tridentata*. f, the same animal as "e" after preservation. Scale bar for all figures is 0.5 cm.

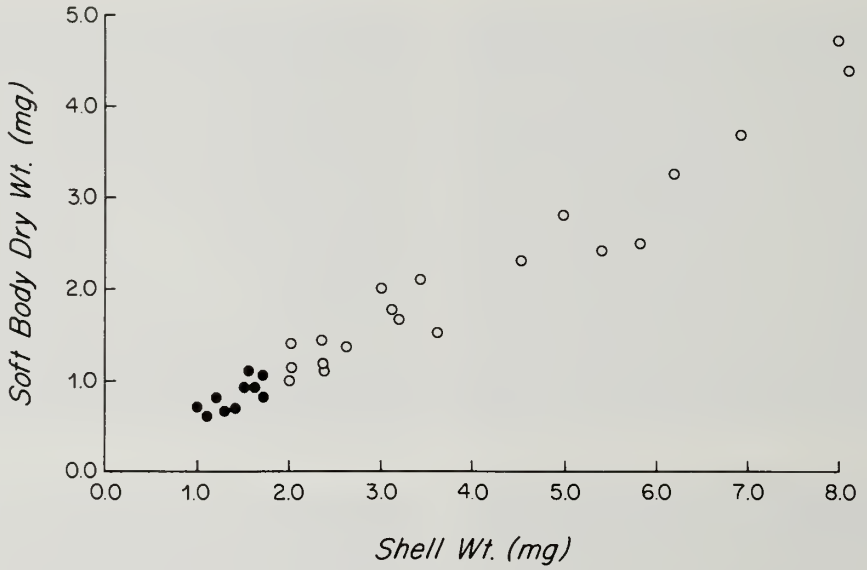

Figure 2

Shell weight to soft-part body weight (dry) for similar dimensions (length, width) of adult-sized specimens of *Clio pyramidata* collected in the western North Atlantic. Shell-wall thickness increases with age and accounts for the differences in shell weight. Solid circles represent preserved specimens showing severe contraction, *i.e.*, the animal occupies less than ⅓ of shell space; open circles are preserved specimens showing little contraction.

weighed separately. Shell dimensions for each specimen of *C. pyramidata* were measured as described by Pafort-van Iersel (1982). Ability to narcotize animals was examined using small amounts of MS-222 (ethyl m-aminobenzoate), ethanol, urethane or menthol crystals, magnesium chloride (osmotic), and by cooling and then preserving animals in 5% buffered formaldehyde.

RESULTS

Most live euthecosome pteropods examined retract violently into their shells when subjected to most common preservatives. They also retract in response to many relaxants if these are added too rapidly. Figure 1a shows a live specimen of a young adult *Clio pyramidata,* while Figure 1b is the same individual after addition of 5% formalin buffered with Borax with no previous narcotization. In Figure 2, all specimens were collected and observed in the living state and were seen to be normally active with their wings well extended. They all had shells of equivalent dimensions in both length (13 ± 0.5 mm) and width (8 ± 0.3 mm) (n = 30). Ten specimens that I preserved became so contracted that the wings and mantle were indistinguishable (Figure 1b). In Figure 2, these 10 contracted specimens composed the entire lower end of the graph, accounting for all of the speicmens with adult shells between 0.95 and 1.7 mg dry weight. Shells of animals used in Figure 2 varied between 0.95 and 8.00 mg in dry weight, but all had identical length-width dimensions. Specimens preserved in formaldehyde for 6 months or less showed no differences in soft-part-to-shell-weight ratios when compared to animals that were only frozen before drying with no preservative added.

In Figure 1c the living specimen of *Cuvierina columnella* (Rang) measured 13 mm in body length when fully extended. In 5% buffered formaldehyde, the soft parts of this animal contracted into a formless mass 3.5 mm in length (Figure 1d) at the bottom of the shell. A second specimen that had similar proportions when alive and was fixed in a similar manner retracted into a "skinny attenuate" animal measuring 8 mm in length inside the shell. Both of these resulting body forms are depicted by Spoel (1967) as supposed living stages of this animal's life cycle. I have similar observations on resulting diminutive body forms in preserved specimens for *Cavolinia tridentata* (Niebuhr) (Figures 1e, f), *C. longirostris* (deBlainville), *C. uncinata* (Rang), *C. gibbosa* (d'Orbigny), *Diacria trispinosa* (deBlainville), *D. quadridentata* (deBlainville), *Creseis virgula* (Rang), *Styliola subula* (Quoy and Gaimard), and *Hyalocylis striata* (Rang), all of which are proposed as having diminutive "aberrant" stages (Spoel, 1967). I was also able to produce "aberrant" specimens like those in Figures 1b, d, and f by adding 10% ethyl alcohol, Bouin's solution, or 2% glutaraldehyde (unbuffered) to unrelaxed specimens.

Results using relaxants varied depending on species, the specimen's age, its condition after collection, and its time in the relaxant. No relaxant I tried prevented severe contraction during fixation in all species. Most species of

Cavolinia and *Creseis,* as well as *Clio pyramidata,* and *Cuvierina columnella* were best relaxed for fixation in a weak solution of MS-222 added a few drops at a time and then left on the specimen in the dark for at least 2 h. Sodium pentobarbitol crystals added in small amounts also prevented severe contraction of *Cavolinia* spp. during fixation but required at least 8 h on the specimen to work. Cooling animals below 5°C is also effective against contraction in all cavoliniids but usually kills them. Ethyl alcohol, menthol or urethane crystals, and magnesium chloride required at least 12 h to show effects and only narcotized animals so that they could be manipulated in the uncontracted state prior to fixation.

DISCUSSION

Juvenile and adult euthecosomes use a large mucous web to entrap food for transport to the mouth (Gilmer & Harbison, in press). This feeding method involves the full extension of the mantle and wings. Thus, in living animals, the wings are always fully developed and capable of great extension beyond the shell regardless of the animal's developmental stage. In most species of cavoliniids, the mantle is only seen at its fullest extension on undisturbed animals observed in the field by SCUBA divers. It is unlikely that these tissues are ever normally contracted or that they regress for a supposed metamorphic or strobilization change. Furthermore, thecosome pteropods have little storage tissue (Baalsrud, 1950; Spoel, 1967), and food particles are present in the guts of all "aberrant" forms (Spoel, 1962, 1967). Because there is no evidence based on observations of living animals that the feeding mechanism changes during the course of a pteropod's development, Spoel's observations can only be explained as fixation artifacts. This is not a problem unique to the shelled forms. The severe contraction of the foot, wings, body, and buccal apparatus is recognized as a major problem in the taxonomy of gymnosomatous pteropods as well (Morton, 1954; Lalli, 1970).

It is evident that animals for which the soft parts are to be used for histology or other descriptive purposes must be examined alive to assess their proportional sizes in relation to the shell size. The extensive mantle tissue seen on living animals became indistinct on all of the preserved specimens of Figure 1 (b, d, f). Other characters such as color or degree of transparency have little meaning in the preserved state without prior knowledge of living animals. Euthecosomes often become opaque and amorphous within minutes of fixation, although the shell may remain transparent for several months in well-buffered fixative.

Several other factors must be considered when working with preserved thecosomes. As is evident from Figure 2, the highly contracted young adult animals occupy shells of the same dimension as older less contractile animals, but differ in the degree of shell weight. This is due to an unusual shell-wall microstructure (Bé *et al.,* 1972) in which new shell material is added to the inner walls over time with very little shell growth at the aperture. Shells of cavoliniids attain the basic form of their maximum dimensions rapidly, and then gradually thicken (Bé *et al.,* 1972; Gilmer, 1974). Therefore, measurements of shell-wall thickness or shell weight will give a better indication of the relative ages of individuals than comparisons of shell dimensions. I have only found diminutive contracted forms when preserving those with the thinnest shells. The heavier and correspondingly older animals do not contract as violently. Variation in the degree that thin-walled specimens contract to form "minute" stages appears to be related to the concentration of the preservative that initially contacts them (*i.e.,* whether or not they lie in the direct path of the fixative when it is added to the sample).

Factors other than preservation artifacts may also contribute to the formation of diminutive soft parts inside adult shells. By means of SCUBA observations, I have seen that thecosomes are the prey of a number of animals, such as gymnosomatous pteropods, hyperiid amphipods, medusae, siphonophores, and ctenophores, that are all capable of removing or digesting all or part of the thecosome body without damaging the shell. Thecosomes are also parasitized by nematodes, pennellid copepods, and probably other amphipods and micro-organisms. These relationships are not well understood but could produce degenerated animals in undamaged shells.

ACKNOWLEDGMENTS

Drs. C. M. Lalli, W. M. Hamner, and G. R. Harbison made valuable comments on the manuscript, which is Woods Hole Oceanographic Institution, Contribution No. 5944. The research was supported by N.S.F. grant OCE-8209341.

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